

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Ryan et al.

Application No.: 10/605,669

Group No.: 1651

Filed: 10/16/2003

Examiner: L.E. Barnhart

For: METHOD AND DEVICE FOR COLLECTING AND PRESERVING CELLS FOR ANALYSIS

Attorney Docket No. 1251.048

Confirmation No.: 2668

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Declaration Under 37 CFR 1.132 of Jodi R. Alt, PhD

1. I am employed by Streck, Inc. as a research scientist. I have been employed in that or a similar capacity for 6 years.
2. I have studied United States Patent Application No. 10/605,669 (the Patent Application).
3. I have reviewed the pending claims of the Patent Application.
4. The pending claims are directed to a cell fixation method that employs what would be regarded by a person of ordinary skill in the art as a concentrated fixative composition wherein a minimal amount of fixative is required to effectively fix a blood sample.
5. Until the present invention, the art of cell fixation traditionally calls for fixatives in large amounts relative to a blood sample. This is for the purpose of avoiding under-fixation to cells that do not contact enough fixative and damage to cells that may contact too much fixative.
6. As further support for the understanding in the art that effective fixing required larger amounts of fixatives in solution so that blood samples are substantially diluted, U.S. Patent No. 5,849,517 to Ryan (as cited against the present application) discloses blood sample to reagent ratios of from about 1:4 to about 2:1, with 1:1 being the

most preferred (see 8:35-38 of Ryan). The blood sample plus fixative to fixative ratio specified in the claims of the present application provides for small amounts of fixative including blood sample to blood sample plus fixative ratios of 100:2; 100:1.5; and 100:1.

7. Based upon this understanding in the art at the time of the present invention, I would expect that the blood sample plus fixative to fixative ratio employed in connection with the pending claims would result in ineffective fixation. I would expect that blood sample to reagent ratios such as those of the claimed method would result in some cells being over-fixed (e.g., shocked) and some cells failing to contact sufficient fixative.
8. However, as represented in the test results attached hereto as **Exhibit A** and **Exhibit B**, the composition of the claimed method shows improved homogeneous cell fixation while avoiding cell damage and/or non-homogeneous fixation as would be expected in light of traditional understanding in the art of cell fixation.
9. I devised a comparative test to compare the fixation ability of various fixation methods in the presently claimed ratio of less than about 2:100 (amount of fixative composition: amount of fixative and blood combined). The fixatives tested include formaldehyde, glutaraldehyde, Streck Cell Preservative¹, and the composition of the claimed invention.
10. Each sample underwent flow cytometric analysis to detect sufficient separation of white blood cell subpopulations. Good white blood cell separation in the subpopulations is necessary to allow for the auto-gating function of the analyzer to be effective. The better the populations are maintained to mimic the whole blood collected in the tube at the time of collection, the fewer problems with gating (due to poor separation and debris) will occur. Additionally, this allows the instrument to properly identify the cells in the whole blood sample.
11. The documents shown at **Exhibit A** show histogram results of blood samples from one donor. Blood from the donor was contacted (in the less than about 2:100 ratio as described in #6 above) with formaldehyde, glutaraldehyde, Streck Cell Preservative, and the composition of the claimed invention.
12. The histogram results at **Exhibit A** show scatter positions of the subpopulations of white blood cells. Improved fixation will show histogram results with readable scatter

¹ Streck Cell Preservative is described in U.S. Patent No. 5,849,517 as cited by the Examiner. Exhibit C includes information identifying that U.S. Patent No. 5,849,517 pertains to the Streck Cell Preservative product.

positions of the white blood cell populations. Poor fixation will result in an inability to differentiate (e.g., per scatter position) the white blood cell subpopulations in the histogram results.

13. As demonstrated at **Exhibit A**, the histogram results for the donor sample contacted with glutaraldehyde fails to produce any readable scatter at day 0.
14. As demonstrated at **Exhibit A**, the histogram results for the donor sample contacted with formaldehyde demonstrates poor scattergram results as of day 5, making it impossible at that time to gate the subpopulations of white blood cells.
15. As shown at **Exhibit A**, based upon evaluation of the day 8 histograms for the composition of the claimed invention as compared to that of the Streck Cell Preservative, the composition of the claimed invention was able to maintain the white blood cell scatter positions needed to provide proper gating of the white blood cell subpopulations. For example, the CD45 lymphocyte population (CD45-PerCP) demonstrates clear separation from debris and other white blood cell subpopulations. The same observation is true for the CD3 (CD3-FITC) population.
16. **Exhibit A** also includes **Table 1**, which shows the change in cell counts from Day 0 to Day 8 (or Day 5 in the case of formaldehyde), comparing donor samples contacted with formaldehyde, Streck Cell Preservative, and the composition of the claimed invention.²
17. As shown in **Table 1**, the resulting cell counts for donor samples contacted with the composition of the present invention with the claimed ratio (2:100) show less change from Day 0 to Day 8 than any donor samples contacted with the Streck Cell Preservative or formaldehyde.
18. I devised an additional comparative test to further compare the fixation ability of the composition of the claimed method and the Streck Cell Preservative in the presently claimed ratio of less than about 2:100 (amount of fixative composition: amount of fixative and blood combined).
19. The documents shown at **Exhibit B** show cell count (as compared to samples contacted with no fixative) and histogram results of blood samples from four donors at day 5 and day 7. Blood from the donors was contacted (in the less than about 2:100 ratio as described in #6 above) with no fixative, Streck Cell Preservative, and the composition of the claimed invention.

² Glutaraldehyde results not shown as analyzed samples produce no counts beyond day 0.

20. As shown at **Exhibit B**, based upon evaluation of the day 5 and day 7 cell counts and histograms for the composition of the claimed invention as compared to that of the Streck Cell Preservative, the composition of the claimed invention was able to maintain the white blood cell scatter positions needed to provide proper gating of the white blood cell subpopulations. For example, on day 5 the CD19 and CD56 CD markers were stable only in the composition of the claimed invention. Further, the absolute counts and percent recoveries on day 7 were unstable in the Streck Cell Preservative as compared to the composition of the claimed invention.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.



Jodi R. Alt, PhD

Dated: Oct 11, 2010

EXHIBIT A

8/10/10

Objective: Demonstrate the importance of the type of fixative and the dilution factor for effective fixation in a ration of about 2:100 (amount of fixative composition: amount of fixative composition plus blood sample).

Procedure: Whole blood from a single donor was collected in K₂EDTA and combined into one pool. 4.9ml of blood was added to each of the following fixative preparations:

1. 100µl of 30% composition of the claimed invention (DU)
2. 100µl of Streck Cell Preserve¹ (IDU)
3. 100µl of 37% formaldehyde solution²
4. 100µl of 25% glutaraldehyde solution³

The samples were analyzed on the FACSCalibur within 2 hours and again after 5 and 8 days at room temperature, except for the glutaraldehyde preservative which did not lyse on day 0.

¹ Streck Cell Preservative is described in U.S. Patent No. 5,849,517 as cited by the Examiner. Exhibit C includes information identifying that U.S. Patent No. 5,849,517 pertains to the Streck Cell Preservative product.

² Standard concentration for formaldehyde fixative solution.

³ Standard concentration for glutaraldehyde fixative solution.

DU

Day 0
1:50 30% DU

STRECK

MultiSET™ Lab Report

Director: DR. RYAN
Operator: Administrator

Software: MultiSET V3.0.1
Cytometer: FACSCalibur (#E97500994)

Sample Name: DU
Sample ID: Day 0
Case Number: Tube 1 Run 1
Panel Name: 4 Color TBNK + TruC

Date Acquired: Wed, Aug 4, 2010 2:26 PM
Date Analyzed: Wed, Aug 4, 2010
Ref. Range Type: BD

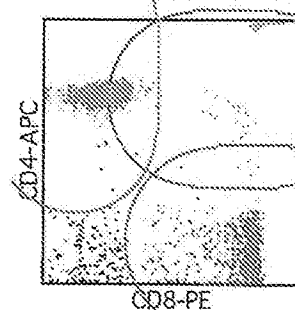
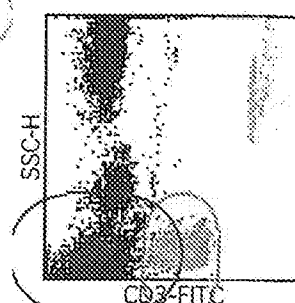
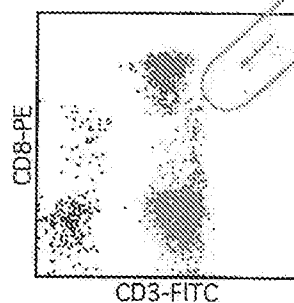
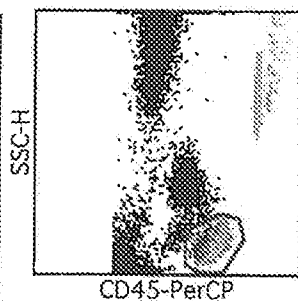
CD3/CD8/CD45/CD4 TruC

Data Set [1] Data File: DU02.01

Reagent Lot ID: 15183 Events Acquired: 15000 Abs Cnt Bd Lot ID: 81093 Attr Def File: 3/8/45/4 MLT/TruC v2.0

File ID: D0134FE6-052B-4D64-
A38F-64EC0D27051A Beads Per Pellet: 50726

Lymph Events	4001
Bead Events	1747
CD3+ %Lymph	78
CD3+ Abs Cnt	1814
CD3+CD8+ %Lymph	28
CD3+CD8+ Abs Cnt	862
CD3+CD4+ %Lymph	43
CD3+CD4+ Abs Cnt	1002
CD3+CD4+CD8+ %Lymph	1
CD3+CD4+CD8+ Abs Cnt	12
CD45+ Abs Cnt	2322
T H/S Ratio	1.51



Day 5 - 22^o
 30% DU - 1:50 dilution **STRECK**

MultiSET™ Lab Report

Director: DR. RYAN
 Operator: Administrator

Software: MultiSET V3.0.1
 Cytometer: FACSCalibur (#E97500994)

Sample Name: DU
 Sample ID: TUBE 1
 Case Number: RUN 1
 Panel Name: 4 Color TBNK + TruC

Date Acquired: Mon, Aug 9, 2010 2:45 PM
 Date Analyzed: Mon, Aug 9, 2010
 Ref. Range Type: BD

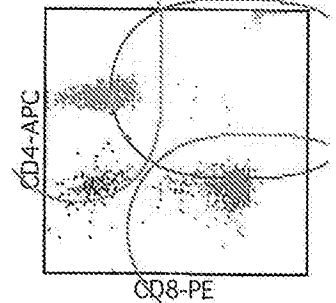
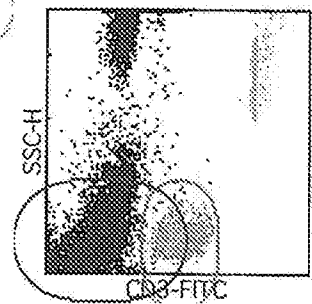
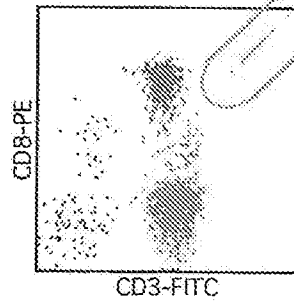
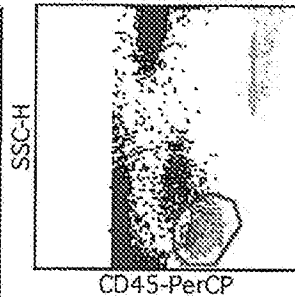
CD3/CD8/CD45/CD4 TruC

Data Set [1] Data File: DU02.01

Reagent Lot ID: 15183 Events Acquired: 15000 Abs Cnt Bd Lot ID: 61093 Attr Def File: 3/8/45/4 MLT/TruC v2.0

File ID: A54EB0B7-301B-4BAC-94A Beads Per Pellet: 50726
 C-7A38D2EE5F05

Lymph Events	2390
Bead Events	1089
CD3+ %Lymph	78
CD3+ Abs Cnt	1739
CD3+CD8+ %Lymph	27
CD3+CD8+ Abs Cnt	606
CD3+CD4+ %Lymph	50
CD3+CD4+ Abs Cnt	1106
CD3+CD4+CD8+ %Lymph	0
CD3+CD4+CD8+ Abs Cnt	9
CD45+ Abs Cnt	2225
T H/S Ratio	1.82



Day 8
1ml Lyse

STRECK MultiSET™ Lab Report

Director: DR. RYAN
Operator: Administrator

Software: MultiSET V3.0.1
Cytometer: FACSCalibur (#E97500994)

Sample Name: DU
Sample ID: DAY 8
Case Number: RUN 1 1 ML LYSE
Panel Name: 4 Color TBNK + TruC

Date Acquired: Thu, Aug 12, 2010 11:20 AM
Date Analyzed: Thu, Aug 12, 2010
Ref. Range Type: BD

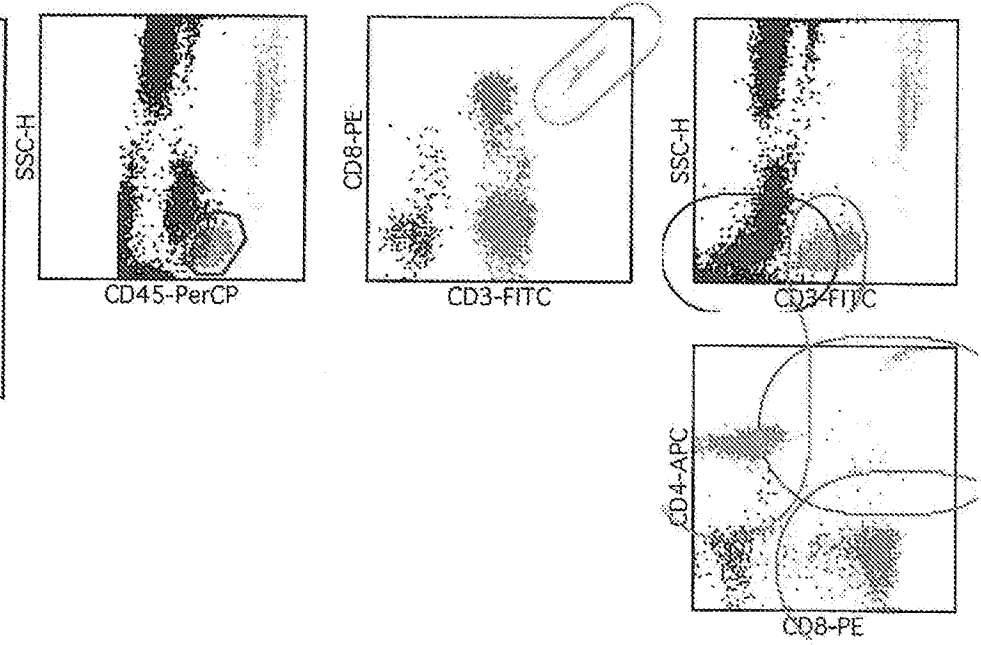
CD3/CD8/CD45/CD4 TruC

Data Set [1] Data File: DU02.01

Reagent Lot ID: 15183 Events Acquired: 15000 Abs Cnt Bd Lot ID: 31920 Attr Def File: 3/8/45/4 MLT/TruC v2.0

File ID: 32CE3603-BBC8-4ADF-
B131-EA8AB5D7CD61 Beads Per Pellet: 50790

Lymph Events	4225
Bead Events	1796
CD3+ %Lymph	79
CD3+ Abs Cnt	1880
CD3+CD8+ %Lymph	27
CD3+CD8+ Abs Cnt	648
CD3+CD4+ %Lymph	44
CD3+CD4+ Abs Cnt	1059
CD3+CD4+CD8+ %Lymph	0
CD3+CD4+CD8+ Abs Cnt	7
CD45+ Abs Cnt	2368
T H/S Ratio	1.64



Day 8
1ml Lyse

Day 0
1:50 5% IDU
cell Preserve

STRECK

MultiSET™ Lab Report

Director: DR. RYAN
Operator: Administrator

Software: MultiSET V3.0.1
Cytometer: FACSCalibur (#E97500994)

Sample Name: IDU
Sample ID: Day 0
Case Number: Tube 1 Run 1
Panel Name: 4 Color TBNK + TruC

Date Acquired: Wed, Aug 4, 2010 2:28 PM
Date Analyzed: Wed, Aug 4, 2010
Ref. Range Type: BD

CD3/CD8/CD45/CD4 TruC

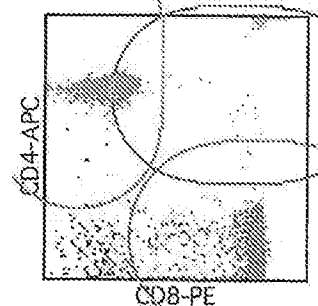
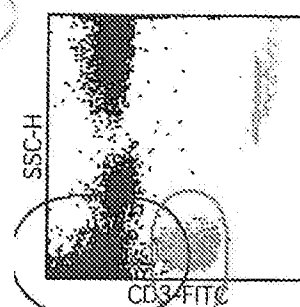
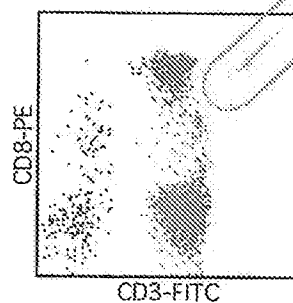
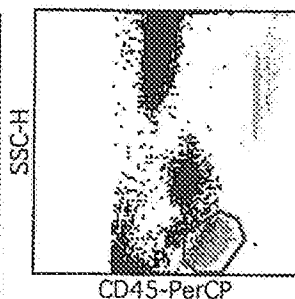
Data Set [1] Data File: IDU03.01

Reagent Lot ID: 15183 Events Acquired: 15000 Abs Cnt Bd Lot ID: 61093 Attr Def File: 3/8/45/4 MLT/TruC v2.0

File ID: BD1BD1B4-
FFF3-4A19-92E4-
A01D064CFCC7

Beads Per Pellet: 50726

Lymph Events	4011
Bead Events	1622
CD3+ %Lymph	78
CD3+ Abs Cnt	1953
CD3+CD8+ %Lymph	28
CD3+CD8+ Abs Cnt	714
CD3+CD4+ %Lymph	42
CD3+CD4+ Abs Cnt	1065
CD3+CD4+CD8+ %Lymph	0
CD3+CD4+CD8+ Abs Cnt	9
CD45+ Abs Cnt	2507
T H/S Ratio	1.49



Day 5-22°
1:50 5% IDU
Cell Preserve

STRECK

MultiSET™ Lab Report

Director: DR. RYAN
Operator: Administrator

Software: MultiSET V3.0.1
Cytometer: FACSCalibur (#E97500994)

Sample Name: IDU
Sample ID: TUBE 1
Case Number: RUN 1
Panel Name: 4 Color TBNK + TruC

Date Acquired: Mon, Aug 9, 2010 2:52 PM
Date Analyzed: Mon, Aug 9, 2010
Ref. Range Type: BD

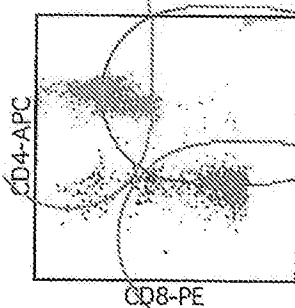
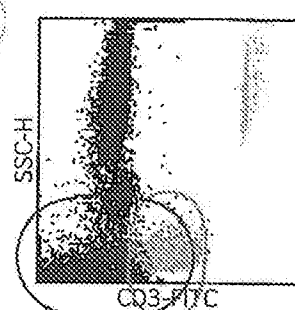
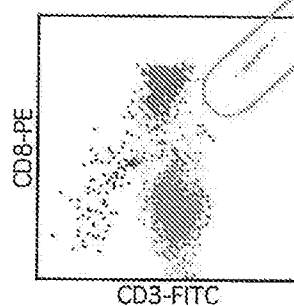
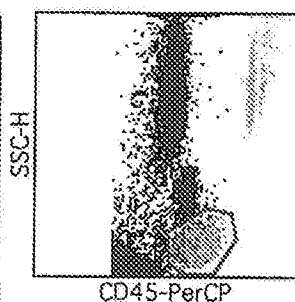
CD3/CD8/CD45/CD4 TruC

Data Set [1] Data File: IDU03.01

Reagent Lot ID: 15183 Events Acquired: 15000 Abs Cnt Bd Lot ID: 61093 Attr Def File: 3/8/45/4 MLT/TruC v2.0

File ID: 3F0029F8-5248-4B0B-9815 Beads Per Pellet: 50726
-8EDF1181A62C

Lymph Events	3240
Bead Events	1397
CD3+ %Lymph	80
CD3+ Abs Cnt	1877
CD3+CD8+ %Lymph	33
CD3+CD8+ Abs Cnt	775
CD3+CD4+ %Lymph	48
CD3+CD4+ Abs Cnt	1132
CD3+CD4+CD8+ %Lymph	4
CD3+CD4+CD8+ Abs Cnt	88
CD45+ Abs Cnt	2352
T H/S Ratio	1.46



STRECK

MultiSET™ Lab Report

Director: DR. RYAN
Operator: Administrator

Software: MultiSET V3.0.1
Cytometer: FACSCalibur (#E97500994)

Sample Name: IDU
Sample ID: DAY 8
Case Number: RUN 1 1 ML LYSE
Panel Name: 4 Color TBNK + TruC

Date Acquired: Thu, Aug 12, 2010 11:22 AM
Date Analyzed: Thu, Aug 12, 2010
Ref. Range Type: BD

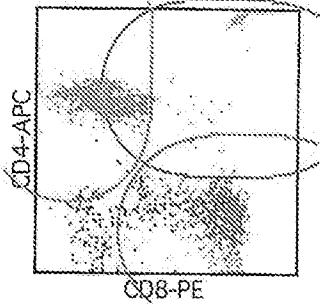
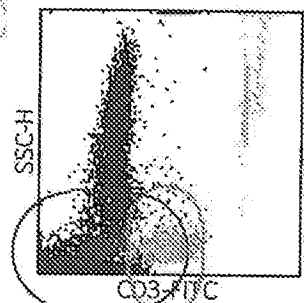
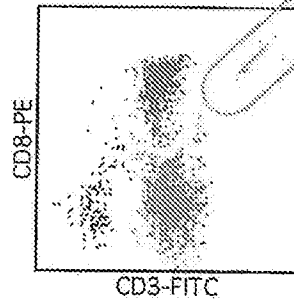
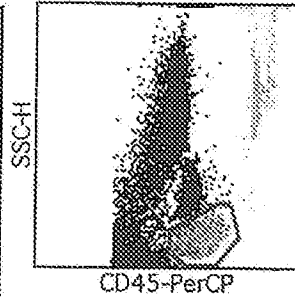
CD3/CD8/CD45/CD4 TruC

Data Set [1] Data File: IDU03.01

Reagent Lot ID: 15183 Events Acquired: 15000 Abs Cnt Bd Lot ID: 31920 Attr Def File: 3/8/45/4 MLT/TruC v2.0

File ID: A0EB4D9C-8F81-4CCB-9B Beads Per Pellet: 50790
73-9C46752F1684

Lymph Events	3006
Bead Events	1443
CD3+ %Lymph	78
CD3+ Abs Cnt	1642
CD3+CD8+ %Lymph	29
CD3+CD8+ Abs Cnt	607
CD3+CD4+ %Lymph	43
CD3+CD4+ Abs Cnt	904
CD3+CD4+CD8+ %Lymph	1
CD3+CD4+CD8+ Abs Cnt	14
CD45+ Abs Cnt	2114
T H/S Ratio	1.49



Day 8
1 ml Lyse

Day 0
1:50 37% Formaldehyde
STRECK

MultiSET™ Lab Report

Director: DR. RYAN
Operator: Administrator

Software: MultiSET V3.0.1
Cytometer: FACSCalibur (#E97500994)

Sample Name: FORM
Sample ID: Day 0
Case Number: Tube 1 Run 1
Panel Name: 4 Color TBNK + TruC

Date Acquired: Wed, Aug 4, 2010 2:30 PM
Date Analyzed: Wed, Aug 4, 2010
Ref. Range Type: BD

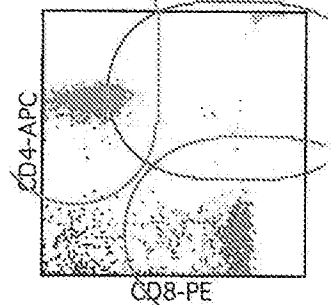
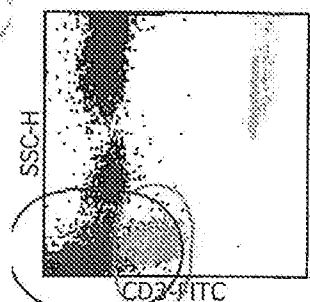
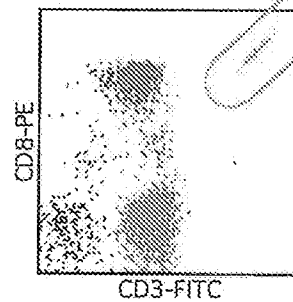
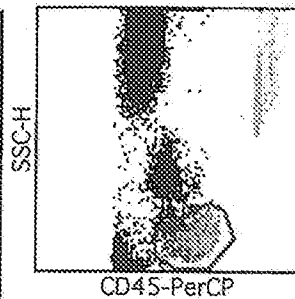
CD3/CD8/CD45/CD4 TruC

Data Set [1] Data File: FORM04.01

Reagent Lot ID: 15183 Events Acquired: 15000 Abs Cnt Bd Lot ID: 61093 Attr Def File: 3/8/45/4 MLT/TruC v2.0

File ID: 33732754-C293-4D62-
A1C4-432AC9A5D2A7 Beads Per Pellet: 50726

Lymph Events	4274
Bead Events	1497
CD3+ %Lymph	77
CD3+ Abs Cnt	2224
CD3+CD8+ %Lymph	28
CD3+CD8+ Abs Cnt	800
CD3+CD4+ %Lymph	42
CD3+CD4+ Abs Cnt	1205
CD3+CD4+CD8+ %Lymph	0
CD3+CD4+CD8+ Abs Cnt	7
CD45+ Abs Cnt	2895
T H/S Ratio	1.51



Day 5 - 22°
1:50 37% formaldehyde
STRECK

MultiSET™ Lab Report

Director: DR. RYAN
Operator: Administrator

Software: MultiSET V3.0.1
Cytometer: FACSCalibur (#E97500994)

Sample Name: FORM
Sample ID: TUBE 1
Case Number: RUN 1
Panel Name: 4 Color TBNK + TruC

Date Acquired: Mon, Aug 9, 2010 2:53 PM
Date Analyzed: Mon, Aug 9, 2010
Ref. Range Type: BD

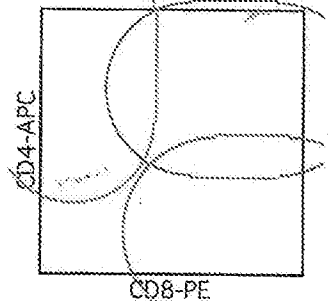
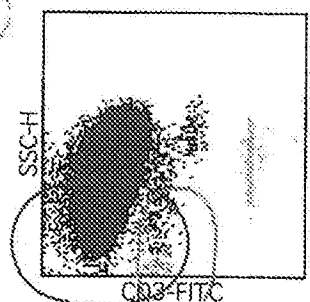
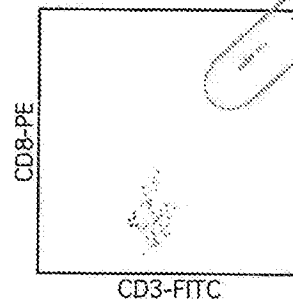
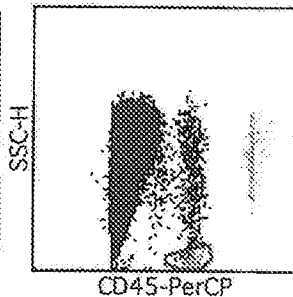
CD3/CD8/CD45/CD4 TruC

Data Set [1] Data File: FORM04.01

Reagent Lot ID: 15183 Events Acquired: 100000 Abs Cnt Bd Lot ID: 61093 Attr Def File: 3/8/45/4 MLT/TruC v2.0

File ID: 2668E6CD-EB94-4995-
A3CA-B538ED6D4551 Beads Per Pellet: 50726

Lymph Events	85
Bead Events	269
CD3+ %Lymph	71
CD3+ Abs Cnt	226 Lo
CD3+CD8+ %Lymph	7 Lo
CD3+CD8+ Abs Cnt	23 Lo
CD3+CD4+ %Lymph	45
CD3+CD4+ Abs Cnt	143 Lo
CD3+CD4+CD8+ %Lymph	0
CD3+CD4+CD8+ Abs Cnt	0
CD45+ Abs Cnt	320
T H/S Ratio	6.33 Hi



QC Messages:

- Code 5: Could not acquire the BDIS strongly recommended 1000 Lymph events.
- Code 6: Could not acquire the BDIS preferred 2000 Lymph events.
- Code 1: Could not acquire the user requested 2000 Lymph events.
- Code 2: Could not acquire the BDIS preferred 500 TruCount Bead events.
- Code 4: The CD3+ Abs Cnt value lies outside the normal reference range.
- Code 4: The CD3+CD8+ %Lymph value lies outside the normal reference range.
- Code 4: The CD3+CD8+ Abs Cnt value lies outside the normal reference range.
- Code 4: The CD3+CD4+ Abs Cnt value lies outside the normal reference range.
- Code 4: The T H/S Ratio value lies outside the normal reference range.

Day 0-22^u
 1:50 25% glutaraldehyde
 STRECK

MultiSET™ Lab Report

Director: DR. RYAN
 Operator: Administrator

Software: MultiSET V3.0.1
 Cytometer: FACSCalibur (#E97500994)

Sample Name: GLUT
 Sample ID: Day 0
 Case Number: Tube 1 Run 1
 Panel Name: 4 Color TBNK + TruC

Date Acquired: Wed, Aug 4, 2010 2:31 PM
 Date Analyzed: Wed, Aug 4, 2010
 Ref. Range Type: BD

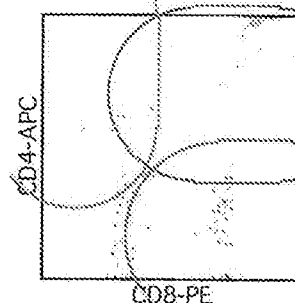
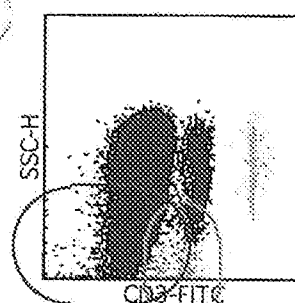
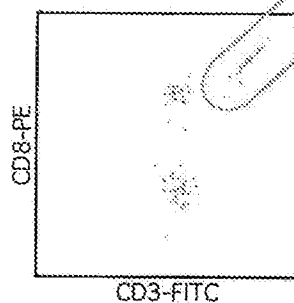
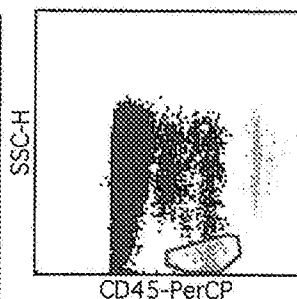
CD3/CD8/CD45/CD4 TruC

Data Set [1] Data File: GLUT05.01

Reagent Lot ID: 15183 Events Acquired: 100000 Abs Cnt Bd Lot ID: 61093 Attr Def File: 3/8/45/4 MLT/TruC v2.0

File ID: 14731491-
 BEE2-44EE-9FD8-676E365
 24547

Lymph Events	139
Bead Events	303
CD3+ %Lymph	99 Hi
CD3+ Abs Cnt	458 Lo
CD3+CD8+ %Lymph	32
CD3+CD8+ Abs Cnt	147 Lo
CD3+CD4+ %Lymph	50
CD3+CD4+ Abs Cnt	231 Lo
CD3+CD4+CD8+ %Lymph	1
CD3+CD4+CD8+ Abs Cnt	7
CD45+ Abs Cnt	465
T H/S Ratio	1.57



QC Messages:

Code 5: Could not acquire the BDIS strongly recommended 1000 Lymph events.
 Code 6: Could not acquire the BDIS preferred 2000 Lymph events.
 Code 1: Could not acquire the user requested 2000 Lymph events.
 Code 2: Could not acquire the BDIS preferred 500 TruCount Bead events.
 Code 4: The CD3+ %Lymph value lies outside the normal reference range.
 Code 4: The CD3+ Abs Cnt value lies outside the normal reference range.
 Code 4: The CD3+CD8+ Abs Cnt value lies outside the normal reference range.
 Code 4: The CD3+CD4+ Abs Cnt value lies outside the normal reference range.

TABLE 1

Claimed composition (DU) v. Streck Cell Preservative (IDU)

	DU Day 0	DU Day 8	change	% change	IDU Day 0	IDU Day 8	change	% change
Lymph events	4001	4225	224	5.6	4011	3006	-1005	25.0
Bead events	1747	1796	39	2.2	1622	1443	-179	11.0
CD3+%lymph	78	79	1	1.2	78	78	0	0
CD3+Abs Cnt	1814	1880	66	3.6	1935	1642	-293	15.1
CD3+CD8+%lymph	28	27	1	3.6	28	29	1	3.6
CD3+CD8+Abs Cnt	662	648	-14	2.1	714	607	-107	15.0
CD3+CD4+%lymph	43	44	1	2.3	42	43	1	2.4
CD3+CD4+Abs Cnt	1002	1059	57	5.7	1065	904	-161	15.1
CD3+CD4+CD8+%lymph	1	0	1	100	0	1	1	100
CD3+CD4+CD8+Abs Cnt	12	7	-5	41.7	9	14	5	55.5
CD45+AbsCnt	2322	2388	66	2.8	2507	2114	-393	15.7

Claimed composition (DU) v. Formaldehyde

	DU Day 0	DU Day 8	change	% change	Formal. Day 0	Formal. Day 5*	change	% change
Lymph events	4001	4225	224	5.6	4274	85	-4189	98.0
Bead events	1747	1796	39	2.2	1497	269	-1228	82.0
CD3+%lymph	78	79	1	1.2	77	71	-6	7.8
CD3+Abs Cnt	1814	1880	66	3.6	2224	226	-1998	89.4
CD3+CD8+%lymph	28	27	1	3.6	28	7	-21	75.0
CD3+CD8+Abs Cnt	662	648	-14	2.1	800	23	-777	97.1
CD3+CD4+%lymph	43	44	1	2.3	42	45	3	7.1
CD3+CD4+Abs Cnt	1002	1059	57	5.7	1205	143	-1062	88.1
CD3+CD4+CD8+%lymph	1	0	1	100	0	0	0	0
CD3+CD4+CD8+Abs Cnt	12	7	-5	41.7	7	0	-7	100
CD45+AbsCnt	2322	2388	66	2.8	2895	321	-2574	98.9

1 Interpretation: Control

2 Data are approximate values

EXHIBIT B

**WHOLE BLOOD STABILITY IN CLAIMED COMPOSITION OF APPLICATION SERIAL NO. 10/605,661
(hereinafter "DU") AS COMPARED TO THE STRECK CELL PRESERVATIVE PRODUCT AS DESCRIBED IN US
PATENT NO. 5,849,517 (hereinafter "IDU")**

Whole Blood Stability in DU vs. IDU

Experimental Design: 4 donor whole blood samples were collected and placed into the formaldehyde-releasing fixatives DU and IDU (both in the ratio of 2:100 (fixative composition to fixative composition plus blood sample)). Sample stability was monitored on the BD FACSCalibur using MultiSet software and MultiTest antibody cocktails after 5 and 7 days at room temperature storage. Relative recoveries of HIV panel markers are shown below.

Acceptance Criteria: CD markers are stable if percent difference is <15% from 6 hour EDTA whole blood evaluation.

Individual Donor Summary--Recoveries on day 5 (n=4 tests)

Absolute Counts						Percent Recovery					
						Outside Acceptance Criteria					
RD1						RD1					
6HR EDTA						6HR EDTA					
	DU	% Diff	IDU	% Diff		DU	% Diff	IDU	% Diff		
	EDTA	EDTA	EDTA	EDTA		EDTA	EDTA	EDTA	EDTA		
CD3	1464	1449	-1	1537	5	86	88	2	97	13	
CD4	796	749	-6	764	-4	47	47	0	47	1	
CD8	525	485	-8	672	28	32	31	-3	42	33	
CD45	1704	1654	-3	1590	-7	97	97	-1	98	1	
CD19	141	119	-16	26	-82	8.0	7.0	-13	1.8	-78	
CD56	53	40	-25	3	-94	3.0	2.7	-11	0.0	-100	
Lymphosum											
RD2						RD2					
6HR EDTA						6HR EDTA					
	DU	% Diff	IDU	% Diff		DU	% Diff	IDU	% Diff		
	EDTA	EDTA	EDTA	EDTA		EDTA	EDTA	EDTA	EDTA		
CD3	2348	2249	-4	2248	-4	84	83	-1	82	-3	
CD4	1574	1538	-2	1564	-1	58	55	-3	56	-3	
CD8	818	657	-8	865	8	23	24	6	24	4	
CD45	2812	2736	-3	2750	-2	98	98	0	95	-3	
CD19	294	296	1	291	-1	10.0	11.3	13	10.5	5	
CD56	129	126	0	79	-39	4.5	4.6	6	3.0	-33	
Lymphosum											
RD3						RD3					
6HR EDTA						6HR EDTA					
	DU	% Diff	IDU	% Diff		DU	% Diff	IDU	% Diff		
	EDTA	EDTA	EDTA	EDTA		EDTA	EDTA	EDTA	EDTA		
CD3	1528	1408	-8	1379	-10	83	83	0	82	-1	
CD4	1035	924	-11	895	-14	55	54	-3	54	-2	
CD8	473	405	-14	409	-14	26	25	-3	25	-4	
CD45	1644	1700	9	1676	9	98	98	0	96	-2	
CD19	190	183	-4	185	-11	10.5	10.5	0	9.8	-7	
CD56	89	96	8	67	-25	4.5	5.2	17	4.0	-11	
Lymphosum											
RD4						RD4					
6HR EDTA						6HR EDTA					
	DU	% Diff	IDU	% Diff		DU	% Diff	IDU	% Diff		
	EDTA	EDTA	EDTA	EDTA		EDTA	EDTA	EDTA	EDTA		
CD3	1586	1468	-7	1672	-1	74	78	3	80	9	
CD4	708	620	-12	666	-6	32	32	0	33	4	
CD8	814	715	-12	821	1	37	37	-1	41	11	
CD45	2156	1948	-10	1967	-9	95	94	-1	96	1	
CD19	304	271	-11	244	-20	14.0	13.8	-2	12.8	-9	
CD56	147	89	-44	69	-53	7.0	4.0	-43	3.3	-54	
Lymphosum											

Summary--Donors 1-4 on day 5

Absolute Counts--Day 5						Percent Recovery--Day 5					
EDTA						EDTA					
6hr						6hr					
	DU	% Diff	IDU	% Diff			DU	% Diff	IDU	% Diff	
	EDTA	EDTA	EDTA	EDTA			EDTA	EDTA	EDTA	EDTA	
CD3	1731	1644	-5	1884	-3	82	82	1	85	4	
CD4	1027	985	-4	971	-5	48	47	-2	48	-1	
CD8	607	565	-7	641	6	29	26	-1	33	12	
CD45	2129	2008	-6	1996	-6	97	97	0	97	-1	
CD19	222	215	-3	182	-21	10.6	10.6	0	8.7	-18	
CD56	104	80	-24	54	-48	4.8	4.3	-10	2.6	-46	
Lymphosum											

Conclusion:

CD19 and CD56 CD markers are not stable in IDU fixative compared to DU fixative.

Individual Donor Summary--Recoveries on day 7 (n=4 tests)

Outside Acceptance Criteria

Absolute Counts

	RD1	DU	% DR	IDU	% DR
	6HR EDTA	EDTA	EDTA	EDTA	EDTA
CD3	1494	1409	-4	1162	-21
CD4	796	759	-5	375	-53
CD8	525	359	-32	294	-44
CD45	1704	1731	2	1684	-1
CD19	141	84	-41	21	-85
CD56	53	17	-67	3	-94

	RD2	DU	% DR	IDU	% DR
	6HR EDTA	EDTA	EDTA	EDTA	EDTA
CD3	2349	2324	-1	2354	0
CD4	1574	1604	2	1626	3
CD8	616	597	-3	693	12
CD45	2612	2620	0	2643	1
CD19	294	316	7	310	5
CD56	129	123	-5	94	-27

	RD3	DU	% DR	IDU	% DR
	6HR EDTA	EDTA	EDTA	EDTA	EDTA
CD3	1526	1429	-6	1299	-15
CD4	1035	921	-11	818	-21
CD8	473	423	-11	393	-17
CD45	1844	1729	-6	1568	-15
CD19	180	179	-6	195	13
CD56	89	93	4	46	-48

	RD4	DU	% DR	IDU	% DR
	6HR EDTA	EDTA	EDTA	EDTA	EDTA
CD3	1585	1413	-11	1495	-6
CD4	703	632	-10	621	-12
CD8	814	712	-13	786	-3
CD45	2156	1695	-13	1825	-15
CD19	304	270	-11	195	-36
CD56	147	80	-46	49	-71

Percent Recovery

	RD1	DU	% DR	IDU	% DR
	6HR EDTA	EDTA	EDTA	EDTA	EDTA
CD3	86	81	-5	88	-23
CD4	47	45	-5	21	-55
CD8	32	21	-34	16	-48
Lymphosum	97	86	-9	89	-9
CD19	8.0	4.6	-41	1.0	-88
CD56	9.0	1.0	-87	0.0	-100

	RD2	DU	% DR	IDU	% DR
	6HR EDTA	EDTA	EDTA	EDTA	EDTA
CD3	84	83	-1	83	-1
CD4	56	57	0	57	-1
CD8	23	23	0	24	6
Lymphosum	98	98	0	97	-1
CD19	10.0	10.6	6	10.8	8
CD56	4.6	4.3	-6	3.0	-33

	RD3	DU	% DR	IDU	% DR
	6HR EDTA	EDTA	EDTA	EDTA	EDTA
CD3	83	83	0	83	0
CD4	55	54	-2	54	-1
CD8	25	25	0	26	1
Lymphosum	98	98	0	98	0
CD19	10.5	10.3	-2	10.3	-2
CD56	4.8	5.0	11	3.0	-33

	RD4	DU	% DR	IDU	% DR
	6HR EDTA	EDTA	EDTA	EDTA	EDTA
CD3	74	75	2	82	11
CD4	32	32	1	34	7
CD8	37	37	1	43	16
Lymphosum	95	95	0	95	0
CD19	14.0	14.3	2	10.8	-23
CD56	7.0	6.0	-14	2.3	-68

Summary--Donors 1-4 on day 7

	EDTA	DU	% DR	IDU	% DR
	6HR EDTA	EDTA	EDTA	EDTA	EDTA
CD3	1731	1644	-5	1577	-9
CD4	1027	979	-5	880	-16
CD8	607	510	-16	542	-11
CD45	2129	2041	-4	1980	-7
CD19	232	212	-9	173	-25
CD56	104	78	-25	47	-55

	EDTA	DU	% DR	IDU	% DR
	6HR EDTA	EDTA	EDTA	EDTA	EDTA
CD3	82	80	-1	79	-3
CD4	46	47	2	42	-13
CD8	29	26	-11	27	-6
Lymphosum	97	95	-2	89	-9
CD19	10.6	10.0	-6	8.2	-23
CD56	4.6	4.1	-14	2.1	-57

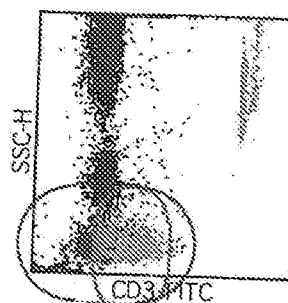
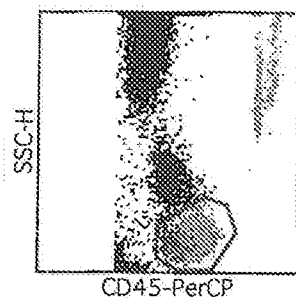
Conclusion:

4-color HIV panel absolute counts and percent recoveries are unstable in IDU vs. DU. Degree of instability can be donor dependent.

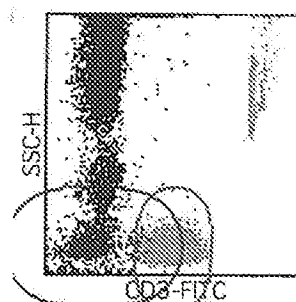
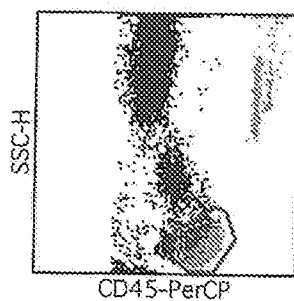
4 Donor Summary--Initial Scans

EDTA Whole Blood

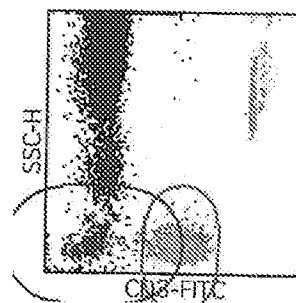
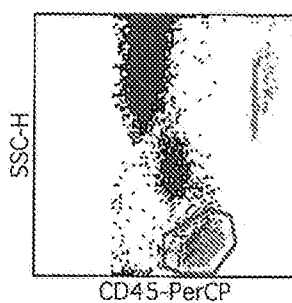
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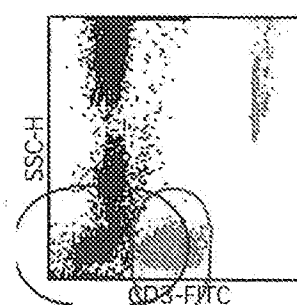
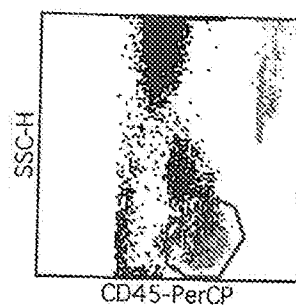
Donor 2



Donor 3



Donor 4

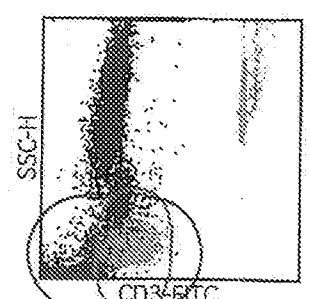
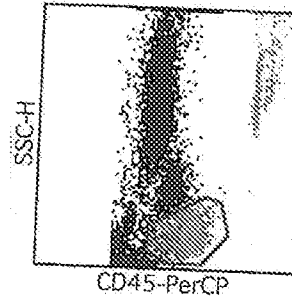
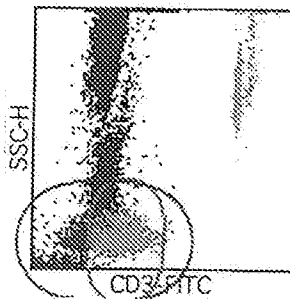
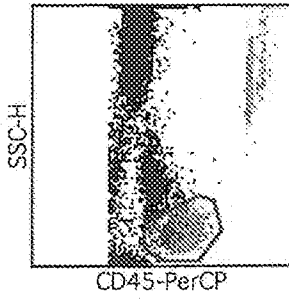


4 Donor Summary--Day 5 Scans

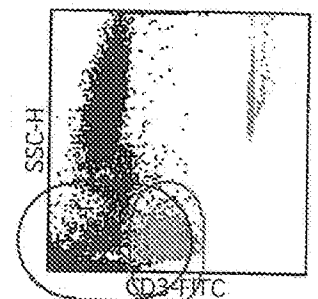
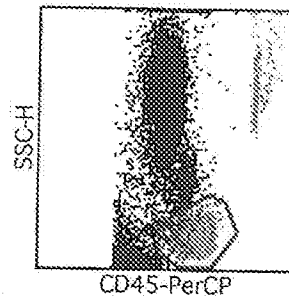
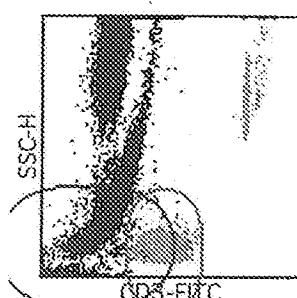
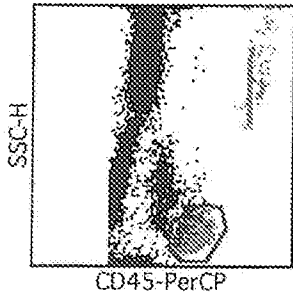
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IDU

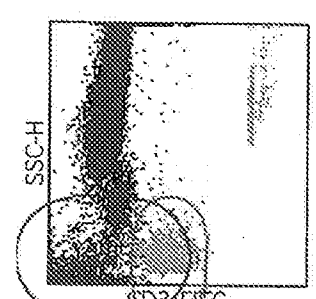
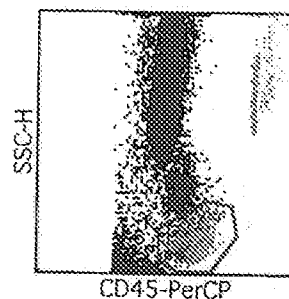
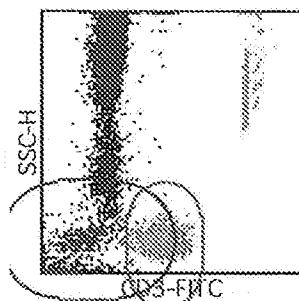
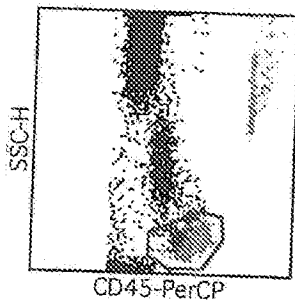
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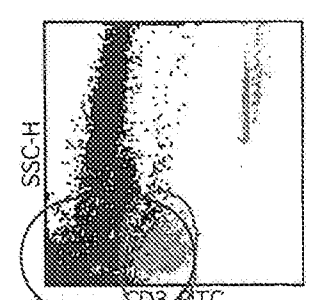
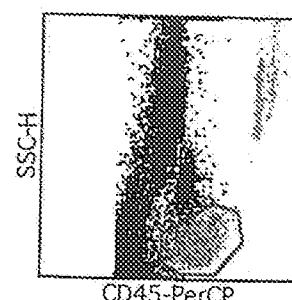
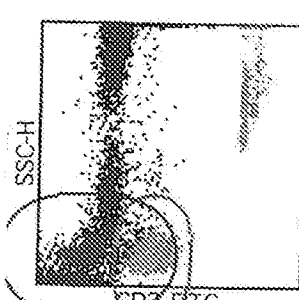
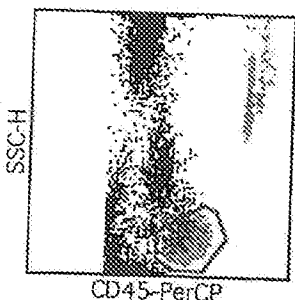
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Donor 3



Donor 4

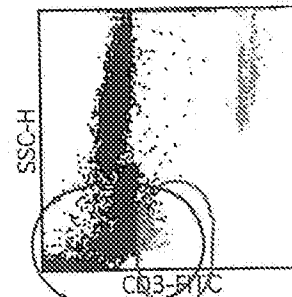
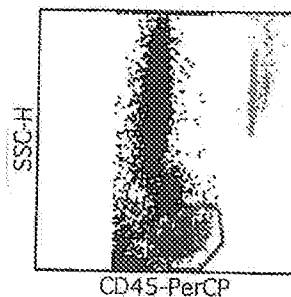
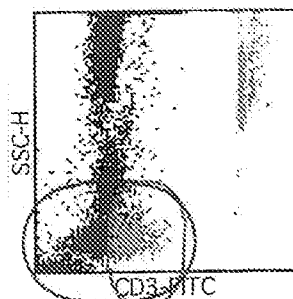
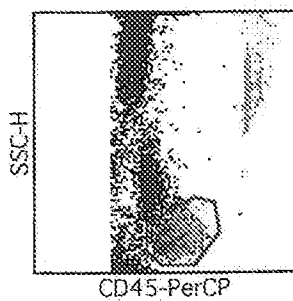


4 Donor Summary--Day 7 Scans

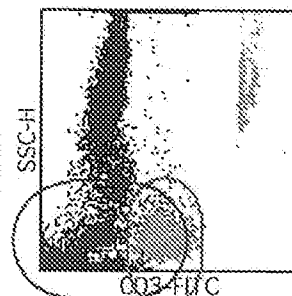
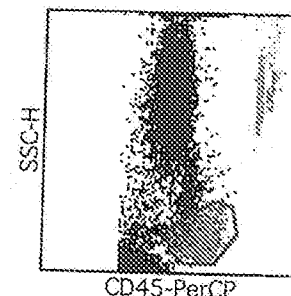
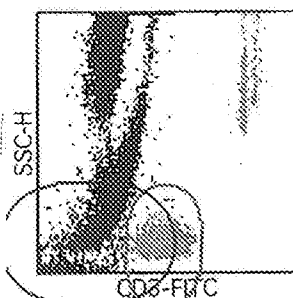
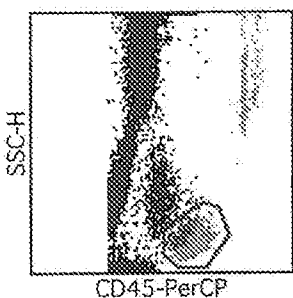
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IDU

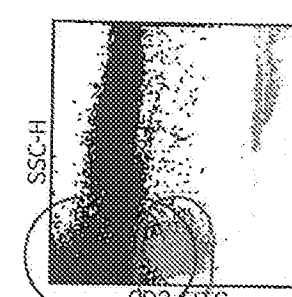
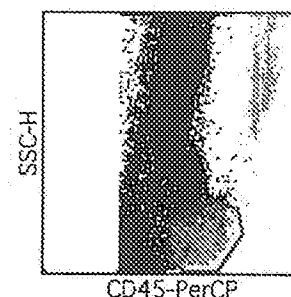
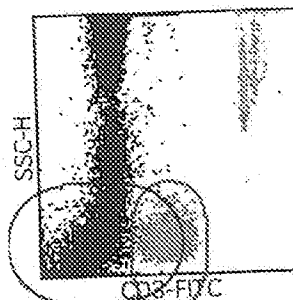
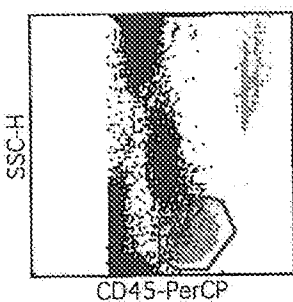
Donor 1



Donor 2



Donor 3



Donor 4

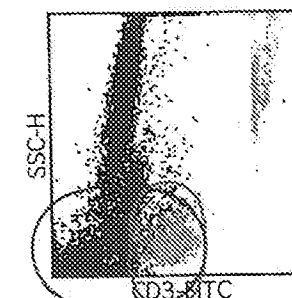
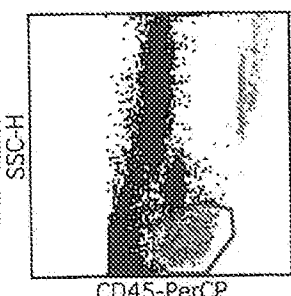
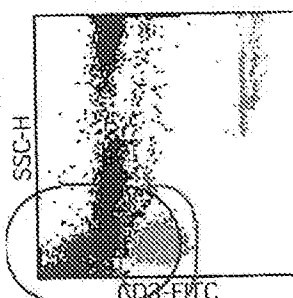
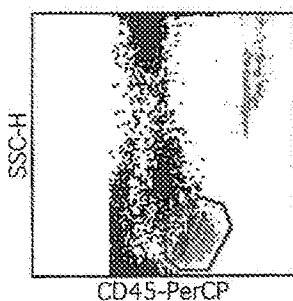


EXHIBIT C



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Streck Cell Preservative

Streck Cell Preservative is a preservative that maintains the integrity of white blood cell antigenic sites. Samples treated with Streck Cell Preservative are stable for up to seven days prior to analysis by flow cytometry, allowing for convenient transport and storage. Streck Cell Preservative offers one-year closed-vial stability.

U.S. Patents 5,156,182; 5,260,048; 5,460,797; 5,459,073; 5,849,517; 5,811,099

Streck Cell Preservative	Catalog No.
6x1.0ml	213350
24x1.0ml	213352
50x1.0ml	213355
2x10ml	213358



Request a
Sample or Catalog »

To place orders, call
Customer Service at 800-
228-6090, or fax your order
to 402-333-6017.

Contact Streck Sales at
800-843-0912 for pricing.

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[Streck Cell Preservative IFU lot 9313, 0085, 0172](#)

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[Streck Cell Preservative Application Note - Bone Marrow Preservation](#)

[Streck Cell Preservative Application Note - Absolute Count Data](#)

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[Cyto-Chex Paper](#)

[Cyto-Chex CD11 Paper](#)

[Poster: Cyto-Chex Reagent Stabilizes Bone Marrow Cells and Their Antigen Expression Profiles for Extended Analysis Using Flow Cytometry](#)



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